pTNMAX (general vector)

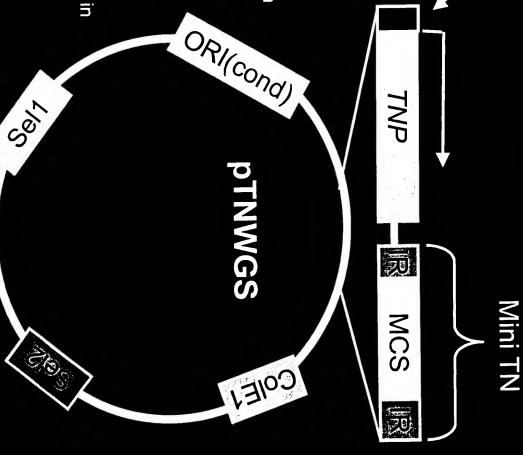
lost specific constitutive promoter

ransposition of the mini TN

ransposition of the minital and its contents.

Ori(cond) conditionally replicative origin of replication for target host organism.

Sel1, 2 markers for selection in cloning and target host organism.



Mini-TN inverted repeats from a transposon active in the target host organism.

MCS multiple cloning site for the introduction of genelibraries into the mini ISS1 transposon.

ORI – origin of replication for the desired cloning hos organism.

pWGS:5

Host specific constitutive promoter.

TN5 Tnp catalyzes the transposition of the mini TN917 and its contents.

Ori-ts temperature sensetive origin of replication for plasmid mainainance in target bacteria (gram positive or negative bacteria).

Mini TN5 TN5 inverted epeach flanking a multiple cloning site into which gene libraries cloned.

CoIE1 origin of replication for plasmid maintainance *E. coli.*

Kanr confers resistar at 10 kanamycin to E. coli

pWGS:917

Mini TN917

Host specific promoter - nisA bacteria. promoter for lactic acid

1917 and its contents transposition of the mini ansposase/resolvase) 1917 TspR TspA catalyzes

tspR tpsA

(dp)

MCS

(()

origin of replication for plasmid bacteria mainainance in Gram positive **PG+** temperature sensetive

erythromycin in Gram positive PG+(ORIS) WITT pTNWGS (Up) COIE

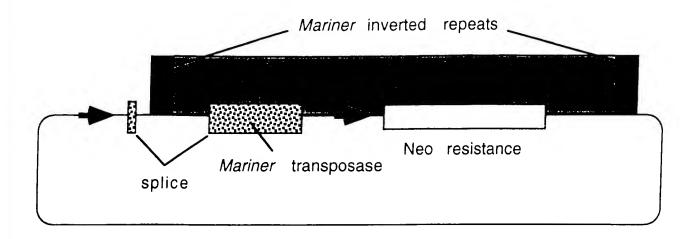
Ermr confers resistance to

the introduction of gene transposon. libraries into the min 11917 MCS multiple cloning site for

plasmid maintenance ColE1 origin of replication for

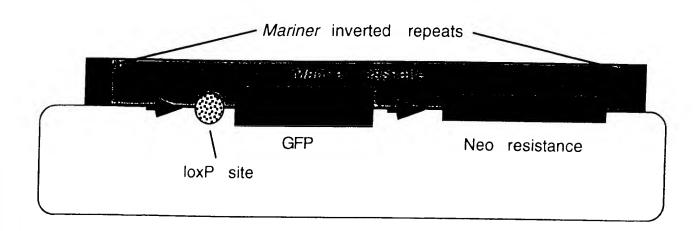
kanamycin to E. col Kanr confers resistand Α

Efficient integration into mammalian cells using evolved *Mariner* transposons

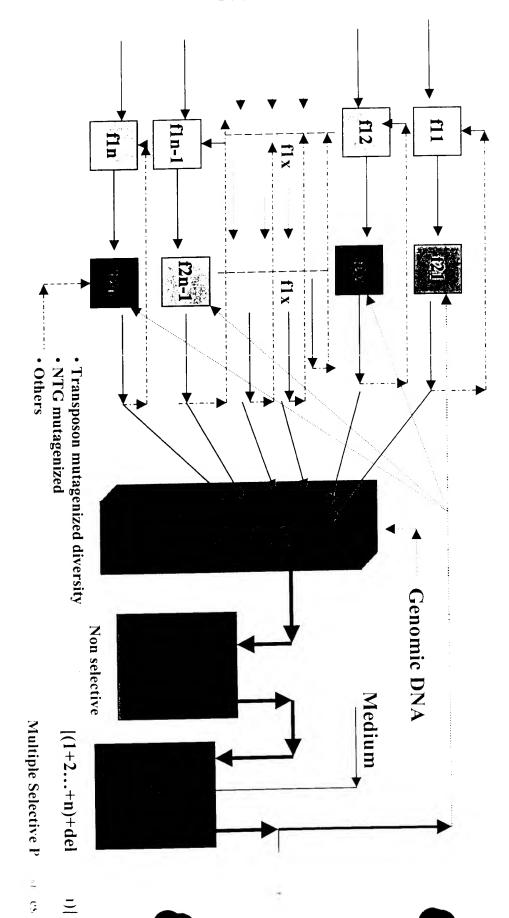


В

Mariner transposon for inserting loxP sites at loci with desirable expression properties

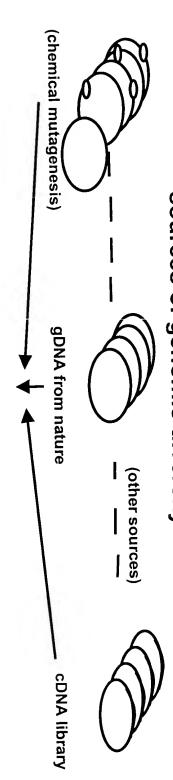


Phenotypes by Whole Genome Shuffling (WGS) Methodology for Isolating Hosts with improved



Shuffling of Genomes *In Vitro:* Formation of transposomes

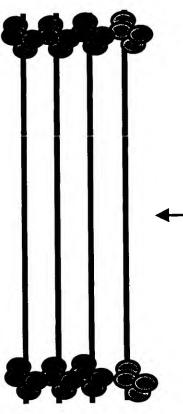
sources of genomic diversity



generate subgenomic fragments
 clone within Tn5 transposon ends

3. add Tn5 transposase

* "transposomes":
complexes poised for
integration upon
exposure to Mg++.



* Complexes are actually circular as a result of transposase interaction

Shuffling of Genomes In Vitro: Breeding multiple donor genomes with a single acceptor genome

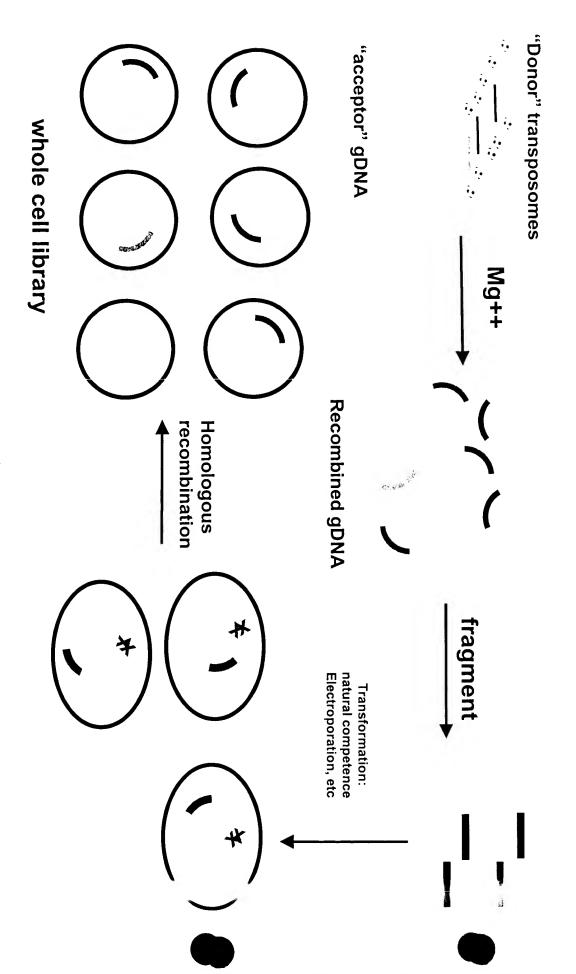


Figure 4B

Shuffling of Genomes In Vitro: Breeding multiple donor genomes with multiple acceptor genomes

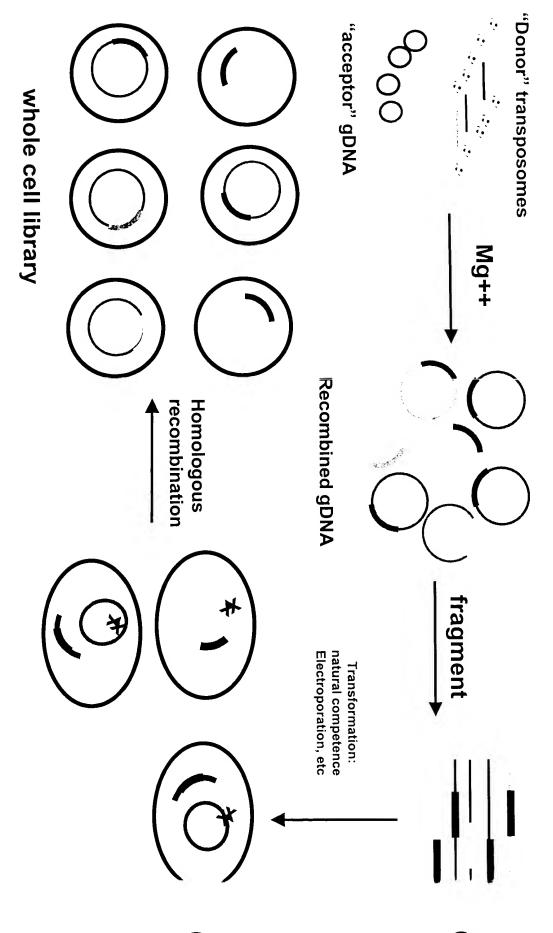


Figure 4C

Split pool recursive in vitro recombination of multiple genomes Shuffling of Genomes In Vitro:

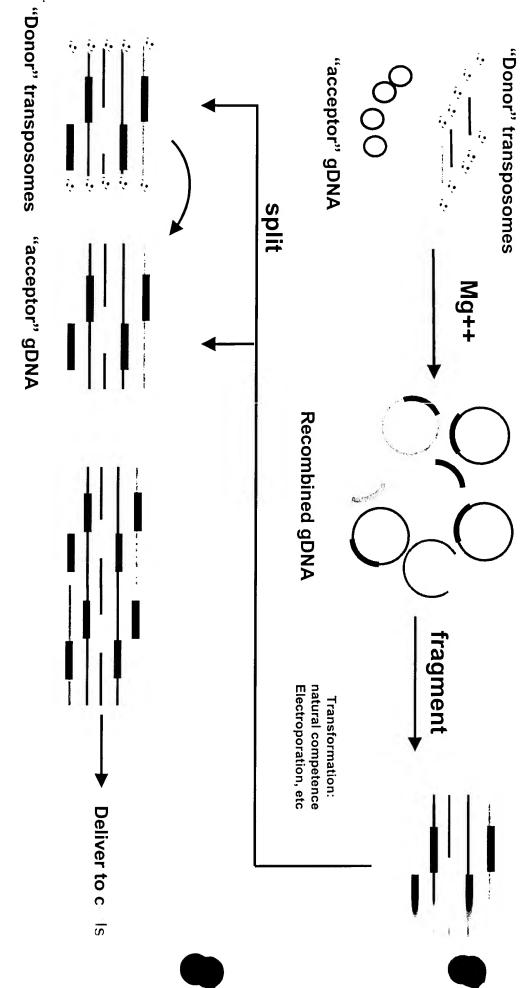


Figure 4D